

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

LISTING OF CLAIMS:

1. (Original) A method for clonal derivation of human blastocyst-derived stem cells (hBS) or hBS derived cells, the method comprising the steps of
 - a) subjecting the hBS cell colonies or hBS derived cell colonies to non-enzymatic treatment to dissociate the cell colonies to one or more single cells,
 - b) selecting/picking of one or more single cells,
 - c) separately cultivating the one or more single cells in a serum based medium and/or serum based conditioned medium,
 - d) optionally, changing the medium to a serum free medium to obtain one or more cell clones capable of forming colonies.
2. (Original) A method according to claim 1, wherein the steps a)-d) are preceded one or more times by the following steps
 - a1) subjecting hBS cell colonies or hBS derived cell colonies to non-enzymatic treatment to dissociate the cell colonies to substantially single cells,
 - b1) selecting/picking of one or more substantially single cells,
 - c1) separately cultivating the one or more substantially single cells in a serum based medium and/or serum based conditioned medium,
 - d1) optionally, changing the medium to a serum free medium to obtain a substantially pure cell population.

3. (Previously Presented) A method according to claim 1, wherein the non-enzymatic method comprises the steps of:

- i) cutting hBS cell colonies of hBS derived cell colonies to obtain smaller units,
- ii) incubating the smaller units with a medium containing a chelator such as, e.g., EDTA,
- iii) triturating the smaller units to obtain hBS single cells or hBS derived single cells.

4. (Original) A method according to claim 3, further comprising a step of
iv) dispersing the hBS single cells or hBS derived single cells in a suitable medium, such as, e.g. a cell free hBS cell conditioned cloning medium (CC-medium), a hBS derived cell free conditioned medium, serum based medium or a hBS culture medium.

5. (Currently Amended) A method according to claim 1, wherein step d)
~~and/or step d1)~~ is included.

6. (Previously Presented) A method according to claim 1, wherein the cell clones obtained in step c) and/or step d) are further cultivated.

7. (Canceled).

8. (Previously Presented) A method according to claim 1, wherein the obtained cell clones are hBS cell clones.

9. (Previously Presented) A method according to claim 1, wherein the hBS derived cells are selected from the group consisting of cells of endodermal, mesodermal, and ectodermal origin.

10. (Previously Presented) A method according to claim 1, wherein the obtained cell clones are selected from the group consisting of cells of endodermal, mesodermal, and ectodermal origin.

11. (Previously Presented) A method according to claim 1, wherein the hBS derived cells are selected from the group consisting of hepatocytes, beta-cells, cardiomyocytes, chondrocytes, osteocytes, keratinocytes, neurons, oligodendrocytes and astrocytes.

12. (Previously Presented) A method according to claim 1, wherein the obtained cell clones are selected from the group consisting of hepatocytes, beta-cells, cardiomyocytes, chondrocytes, osteocytes, keratinocytes, neurons, oligodendrocytes and astrocytes.

13. (Currently Amended) A method according to claim 1, wherein the cultivation in step c) and/or c1) is performed in a medium that promotes propagation of the one or more hBS cells or hBS derived cells.

14. (Currently Amended) A method according to claim 1, wherein step c) and/or ~~step c1~~) is performed in the presence of fibroblasts.

15. (Currently Amended) A method according to claim 1, wherein step c) and/or ~~step c1~~) is performed under feeder cell free conditions.

16. (Previously Presented) A method according to claim 15, wherein step c) and/or ~~step ci~~) is performed on a support substrate comprising a component that promotes colony formation and/or cell division and/or adhesion and/or inhibits differentiation of the hBS single cells or hBS derived single cells.

17. (Canceled).

18. (Canceled).

19. (Currently Amended) A method according to claim 14, wherein said fibroblasts are selected from the group consisting of mouse embryonic fibroblasts, human foreskin fibroblasts, fetal skin fibroblasts, fetal muscle fibroblast fibroblasts, adult skin fibroblasts and fibroblasts derived from hBS cells.

20. (Previously Presented) A method according to claim 16, wherein said component that promotes colony formation and/or cell division and/or adhesion and/or inhibits differentiation of the hBS cells or hBS derived cells is selected from

the group consisting of albumin, gelatine, poly-ornithine, fibronectin, vitronectin, agarose, poly-L-lysine, collagen, an extracellular matrix component, and combinations of the foregoing.

21. (New) A method for clonal derivation of human blastocyst-derived stem cells (hBS) or hBS derived cells, the method comprising the steps of

- a) subjecting the hBS cell colonies or hBS derived cell colonies to non-enzymatic.
- b) selecting/picking of one or more single cells,
- c) separately cultivating the one or more single cells in a serum based medium and/or serum based conditioned medium.
- d) optionally, changing the medium to a serum free medium to obtain one or more cell clones capable of forming colonies, the steps a)-d) being preceded one or more times by the following steps
 - a1) subjecting the hBS cell colonies or hBS derived cell colonies to non-enzymatic treatment to disassociate the cell colonies to substantially single cells.
 - b1) selecting/picking of one or more substantially single cells,
 - c1) separately cultivating the one or more substantially single cells in a serum based medium and/or serum based conditioned medium,
 - d1) optionally, changing the medium to a serum free medium to obtain a substantially pure cell population.

22. (New) A method according to claim 21, wherein the non-enzymatic method comprises the steps of

- i) cutting hBS cell colonies of hBS derived cell colonies to obtain smaller units,
- ii) incubating the smaller units with a medium containing a chelator such as, e.g., EDTA, and
- iii) triturating the smaller units to obtain hBS single cells or hBS derived single cells.

23. (New) A method according to claim 22, further comprising a step of iv) dispersing the hBS single cells or hBS derived single cells in a suitable medium, such as, e.g., a cell free hBS cell conditioned cloning medium (CC-medium), a hBS derived cell free conditioned medium, serum based medium or a hBS culture medium.

24. (New) A method according to claim 21, wherein step d) and/or step d1) is included.

25. (New) A method according to claim 21, wherein the cell clones obtained in step c) and/or step d) are further cultivated.

26. (New) A method according to claim 21, wherein the substantially pure cell populations obtained in step c1) and/or step d1) are further cultivated.

27. (New) A method according to claim 21, wherein the obtained cell clones are hBS cell clones.

28. (New) A method according to claim 21, wherein the hBS derived cells are selected from the group consisting of cells of endodermal, mesodermal, and ectodermal origin.

29. (New) A method according to claim 21, wherein the obtained cell clones are selected from the group consisting of cells of endodermal, mesodermal, and ectodermal origin.

30. (New) A method according to claim 21, wherein the hBS derived cells are selected from the group consisting of hepatocytes, beta-cells, cardiomyocytes, chondrocytes, osteocytes, keratinocytes, neurons, oligodendrocytes and astrocytes.

31. (New) A method according to claim 21, wherein the obtained cell clones are selected from the group consisting of hepatocytes, beta-cells, cardiomyocytes, chondrocytes, osteocytes, keratinocytes, neurons, oligodendrocytes and astrocytes.

32. (New) A method according to claim 21, wherein the cultivation in step c) and/or c1) is performed in a medium that promotes propagation of the one or more hBS cells or hBS derived cells.

33. (New) A method according to claim 21, wherein step c) and/or step c1) is performed in the presence of fibroblasts.

34. (New) A method according to claim 21, wherein step c) and/or step c1) is performed under feeder cell free conditions.

35. (New) A method according to claim 34, wherein step c) and/or step c1) is performed on a support substrate comprising a component that promotes colony formation and/or cell division and/or adhesion and/or inhibits differentiation of the hBS single cells or hBS derived single cells.

36. (New) A method according to claim 33, wherein said fibroblasts are selected from the group consisting of mouse embryonic fibroblasts, human foreskin fibroblasts, fetal skin fibroblasts, fetal muscle fibroblasts, adult skin fibroblasts and fibroblasts derived from hBS cells.

37. (New) A method according to claim 35, wherein said component that promotes colony formation and/or cell division and/or adhesion and/or inhibits differentiation of the hBS single cells or hBS derived single cells is selected from the group consisting of albumin, gelatine, poly-ornithine, fibronectin, vitronectin, agarose, poly-L-lysine, collagen, an extracellular matrix component, and combinations of the foregoing.